

# Theoretical and Experimental Dissection of Gravity-Dependent Mechanical Orientation in Gravitactic Microorganisms

YOSHIHIRO MOGAMI,\* JUNKO ISHII,† AND SHOJI A. BABA

*Department of Biology, Ochanomizu University, Otsuka, Tokyo 112-8610, Japan*

**Abstract.** Mechanisms of gravitactic behaviors of aquatic microorganisms were investigated in terms of their mechanical basis of gravity-dependent orientation. Two mechanical mechanisms have been considered as possible sources of the orientation torque generated on the inert body. One results from the differential density within an organism (the gravity-buoyancy model) and the other from the geometrical asymmetry of an organism (the drag-gravity model). We first introduced a simple theory that distinguishes between these models by measuring sedimentation of immobilized organisms in a medium of higher density than that of the organisms.  $\text{Ni}^{2+}$ -immobilized cells of *Paramecium caudatum* oriented downwards while floating upwards in the Percoll-containing hyper-density medium but oriented upwards while sinking in the hypo-density control medium. This means that the orientation of *Paramecium* is mechanically biased by the torque generated mainly due to the anterior location of the reaction center of hydrodynamic stress relative to those of buoyancy and gravity; thus the torque results from the geometrical fore-aft asymmetry and is described by the drag-gravity model. The same mechanical property was demonstrated in gastrula larvae of the sea urchin by observing the orientation during sedimentation of the KCN-immobilized larvae in media of different density: like the paramecia, the gastrulae oriented upwards in hypo-density medium and downwards in hyper-density medium. Immobilized pluteus larvae, however, oriented upwards regardless of the density of the medium. This indicates that the orientation of the pluteus is biased by the torque generated mainly due to the posterior location of the reaction center of gravity relative to those of buoyancy and hydro-

dynamic stress; thus the torque results from the fore-aft asymmetry of the density distribution and is described by the gravity-buoyancy model. These observations indicate that, during development, sea urchin larvae change the mechanical mechanism for the gravitactic orientation. Evidence presented in the present paper demonstrates a definite relationship between the morphology and the gravitactic behavior of microorganisms.

## Introduction

Many swimming microorganisms, including ciliate and flagellate protozoa and the planktonic larvae of some invertebrates, are negatively gravitactic; that is, they tend to swim preferentially upwards in water columns despite being heavier than water. This behavior requires the organism to orient upwards in relation to the gravity vector. Several mechanisms have been postulated for the gravitactic orientation of aquatic microorganisms (Chia *et al.*, 1983; Bean, 1984; Machemer and Bräucker, 1992). From a physical point of view and taking account of the mechanical properties of these microorganisms, it has been postulated that the interaction of gravitational and hydrodynamic forces may cause them to orient with fore end upward. In addition to the mechanical basis, gravitactic orientation might also be explained on the physiological basis of gravity perception. To modulate the propulsive activity, some mechanosensitive devices that sense gravity (for example, statocysts) might be needed. Although functional statocysts have been found in some unicellular organisms (Fenchel and Finlay, 1984, 1986), a line of evidence for gravity-dependent modulation of propulsion has been accumulated for *Paramecium* (Machemer *et al.*, 1991; Ooya *et al.*, 1992) and *Euglena* (Machemer-Röhnisch *et al.*, 1999), which have no statocyst-like structure.

The present paper focuses on the mechanical properties of

Received 14 July 2000; accepted 30 March 2001.

\* To whom correspondence should be addressed. E-mail: mogami@cc.ocha.ac.jp

† Died on 1 March 1999.

microorganisms, which, irrespective of propulsion, generate the torque to orient the organisms either upwards or downwards. This mechanical torque should bias the gravitactic orientation, even if the organisms have active physiological mechanisms of gravitaxis. According to Roberts (1970), two mechanical mechanisms have been considered as possible sources of the orientation torque. These are reconsidered, in the present paper, as two mechanical models, the gravity-buoyancy model and the drag-gravity model.

The gravity-buoyancy model was first postulated by Verworn (1889, cited in Machemer and Bräucker, 1992) for the negative gravitaxis of *Paramecium*. This model is based on the differential density within an organism. If the internal density of the organism is not homogeneous, the center of mass (the center of gravity) does not necessarily coincide with the centroid (the center of buoyancy). Posterior accumulation of the mass would result in the upward orientation of the organisms, and anterior accumulation would result in the downward orientation.

The drag-gravity model was postulated by Roberts (1970) on the basis of the low Reynolds number hydrodynamics of the swimming of microorganisms that have a geometrical fore-aft asymmetry. This model is characterized by a dumbbell with two spheres of unequal diameter but homogeneous density, which could mimic the fore-aft asymmetry of the microorganisms. According to Stokes' drag formula, the larger sphere of the dumbbell can sink faster than the smaller, at the rate of the square of the ratio of diameters. The applicability of this model has been confirmed by scale-model experiments (Roberts, 1970).

Organisms, in general, possess some asymmetry both in internal density and in external geometry. It is therefore possible that these two mechanical models operate independently to generate the gravity-induced orientation torque. Since the mechanical properties for gravitactic orientation are independent of propulsive thrust, we can assess the mechanical influence by measuring the orientation of immobilized organisms sinking under gravity. Both models predict that, when immobilized, an organism orients upwards when sinking in a medium with a density lower than its own.

In the present paper, we show that the above two models can be distinguished by observing what happens to an organism placed in a medium whose density is higher than its own. We show the results of the experiments on the gravitactic orientation of *Paramecium* and sea urchin larvae, both of which are known to perform typical negative gravitaxis (Mogami *et al.*, 1988; Ooya *et al.*, 1992).

## Theory

The external forces acting on the body of an aquatic microorganism due to gravity acceleration are gravitational ( $F_G$ ) and buoyant forces ( $F_B$ ), each of which is generated as the product of the volume and density of the body or of the external fluid. The vector sum of the forces encounters the hydrodynamic

force ( $F_H$ ). Since the Reynolds number of an aquatic microorganism in translational motion is significantly less than unity (of the order of  $10^{-2}$ ),  $F_H$  is generated in proportion to the velocity (Happel and Brenner, 1973; Vogel, 1994).  $F_G$ ,  $F_B$ , and  $F_H$  act on the center of mass ( $G$ ), the centroid ( $B$ ), and the reaction center of hydrodynamic stress ( $H$ ), respectively. For an immobilized microorganism sinking in the fluid, these three forces are balanced as

$$F_G + F_B + F_H = 0. \quad (1)$$

Each term in the equation (positive in upward direction) is described as

$$F_G = -V\rho_i g, \quad (2)$$

$$F_B = V\rho g, \text{ and} \quad (3)$$

$$F_H = -Ks, \quad (4)$$

where  $V$  and  $\rho_i$  are the total volume and the average density of the organism,  $\rho$  and  $g$  the density of the external fluid and the acceleration due to gravity, and  $K$  and  $s$  the coefficient of hydrodynamic drag and the sinking velocity.

We assume in the present paper that a microorganism has a body of rotating symmetry on its fore-aft axis. The simplest case of this approximation is that the body has fore-aft symmetry, such as a prolate spheroid. When a prolate spheroid with uniform density is sinking in the fluid, the three forces act on the same point and therefore do not generate any torque to rotate the body (Fig. 1a).

If, however, the body of a prolate spheroid has a region of higher density in the rear half of the body, as postulated in the gravity-buoyancy model,  $G$  is located posterior to  $B$  and  $H$  (Fig. 1b). This generates the torque ( $T_V$ ; subscript  $V$  is after Verworn) which is given by

$$T_V = F_G L_G \sin \theta, \quad (5)$$

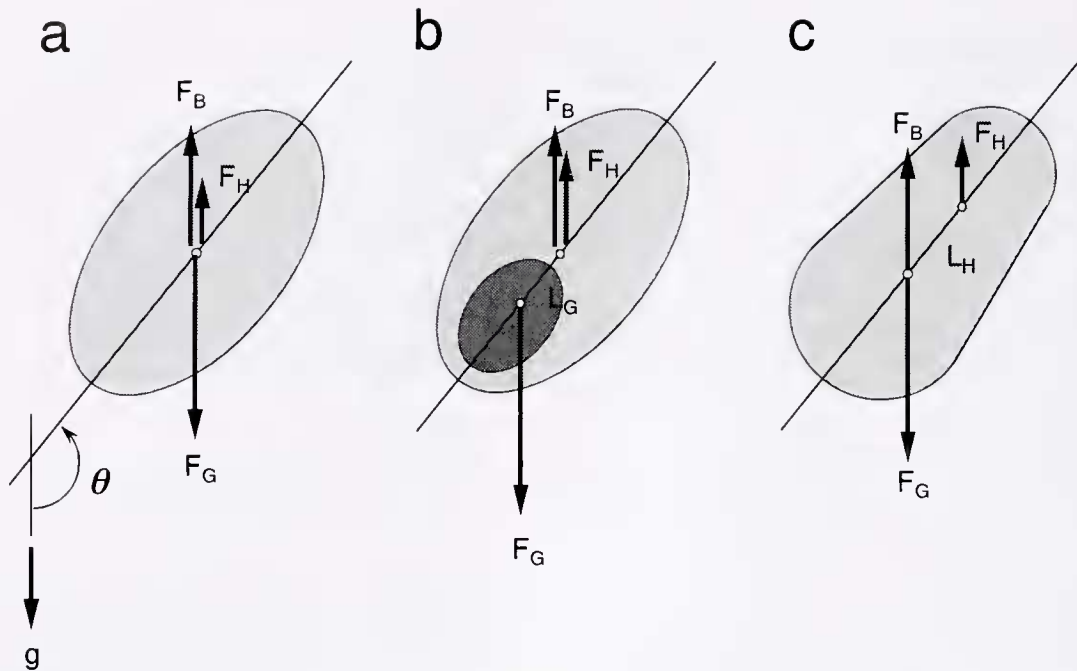
where  $L_G$  is the distance between  $G$  and  $B$  (and/or  $H$ ), and  $\theta$  is the orientation angle of the fore-aft axis of the body to the vertical.

The fore-aft asymmetry of the external geometry, as postulated in the drag-gravity model, also separates the reaction centers of the forces. If a microorganism of homogeneous density has a larger radius of revolution around the fore-aft axis in the posterior part (Fig. 1c),  $H$  is located anterior to  $B$  and  $G$ , according to the analogy of a fore-aft asymmetrical dumbbell of homogeneous density (Happel and Brenner, 1973). The torque ( $T_R$ ; subscript  $R$  is after Roberts) by the anterior shift of the center of hydrodynamic force is given by

$$T_R = -F_H L_H \sin \theta = (F_G + F_B) L_H \sin \theta, \quad (6)$$

where  $L_H$  is the distance between  $H$  and  $G$  (and/or  $B$ ).

Provided that the Reynolds number of rotational motion is sufficiently small, all torques should be proportional to



**Figure 1.** Schematic drawings illustrating the mechanical (physical) basis for the generation of gravity-dependent orientation torque. Gravity ( $F_G$ ), buoyancy ( $F_B$ ), and hydrodynamic force ( $F_H$ ) are balanced in sinking microorganisms; these forces act at the center of mass ( $G$ ), the centroid ( $B$ ), and the reaction center of hydrodynamic stress ( $H$ ), respectively. (a) Three forces act at the same point in the body of prolate spheroid with uniform density. (b) The center of mass is deviated to the rear end of the body of prolate spheroid, which generates the torque in proportion to  $F_G$  and the sine of the orientation angle to the gravity vector ( $\theta$ ). (c) The reaction center of hydrodynamic stress is deviated to the front end of the body with fore-aft asymmetry but with uniform density, which generates the torque in proportion to the vector sum of  $F_G$  and  $F_B$  and the sine of the orientation angle.

the first power of rotational velocity ( $d\theta/dt$ ). In such cases equations of rotational motion are given by

$$-R\eta \frac{d\theta}{dt} = T_V \text{ or } T_R, \quad (7)$$

where  $R$  is the coefficient of resistance for rotational motion and  $\eta$  is the viscosity of the external fluid. From these equations the rotational velocity of each model is given as a common form of

$$\frac{d\theta}{dt} = \beta \sin \theta, \quad (8)$$

where the proportional factor is the instantaneous rate at  $\theta = 90$  degrees, and given by

$$\beta = \beta_V = \frac{V\rho_i g L_G}{R\eta} \text{ and} \quad (9)$$

$$\beta = \beta_R = \frac{V(\rho_i - \rho) g L_H}{R\eta}, \quad (10)$$

for the gravity-buoyancy and drag-gravity models, respectively.

Equations 9 and 10 indicate that  $\beta_V$  is insensitive to

changes in the density of the external medium ( $\rho$ ), whereas  $\beta_R$  reverses the sign as  $\rho$  exceeds the density of organisms ( $\rho_i$ ). This means that the two models can be distinguished by increasing  $\rho$  greater than  $\rho_i$ . When immobilized organisms are immersed in the hyper-density medium ( $\rho > \rho_i$ ), they would orient upwards during floating upwards if they obeyed the gravity-buoyancy model, whereas they would orient downwards if they obeyed the drag-gravity model.

The gravity-buoyancy and drag-gravity models are the two extremes of these conditions that can generate the orientation torque depending on the different physical mechanisms. Passive orientation of the organisms (Eq. 8), in fact, would be explained as a result of combining the two models, because none of three forces would necessarily have a common reaction center. In order to extract the origin of the mechanical bias of the orientation, Equation 8 should be examined by measuring  $\beta$  by the sedimentation experiment using media of different  $\rho$ . If  $\beta$  is constant independent of  $\rho$ , the gravity-buoyancy model is the only mechanism for generating the orientation torque. Otherwise, the drag-gravity model may play a part in the generation of the torque. A negative value of  $\beta$  in the hyper-density medium indicates

that the drag-gravity model is the major mechanism in passive gravitactic orientation.

## Materials and Methods

### *Microorganisms and experimental solutions*

*Paramecium caudatum* was grown at 24 °C in a hay infusion in Dryl's solution (2 mM sodium citrate, 1.2 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.0 mM NaH<sub>2</sub>PO<sub>4</sub>, 1.5 mM CaCl<sub>2</sub>, pH 7.2). Cells grown to the early stationary phase (14–20 d after incubation) were collected and adapted in the experimental solution (KCM: 1.0 mM KCl, 1.0 mM CaCl<sub>2</sub>, 1.0 mM MOPS, pH 7.2). After the adaptation, cells gravitactically accumulating beneath the water surface were collected and immobilized in the KCM containing 5 mM NiCl<sub>2</sub>. Hyper-density KCM (P-KCM) was prepared by substituting a colloidal solution of Percoll (Sigma) for water up to 60% (v/v) in KCM. At 24 °C, the specific gravity and relative viscosity of KCM were 1.00 and 1.02, respectively; those of P-KCM were 1.06 and 1.57. Specific gravity of the experimental solutions was determined by weighing the known volume, and viscosity was measured by means of an Ostwald viscometer.

Larvae of the sea urchin *Hemicentrotus pulcherrimus* were grown in the laboratory at 17 °C (Degawa *et al.*, 1986). Larvae at the mid- to late gastrula stage and the early pluteus stage (*ca.* 24 and 48 h after insemination, respectively) were collected by hand centrifuge and washed once with artificial seawater (ASW; 450 mM NaCl, 10 mM KCl, 10 mM CaCl<sub>2</sub>, 25 mM MgCl<sub>2</sub>, 28 mM MgSO<sub>4</sub>, 10 mM Tris-HCl, pH 8.0). For immobilization, larvae were immersed in ASW containing 2 mM KCN. Hyper-density ASW (P-ASW) was prepared by substituting Percoll for water up to 22% (v/v) in ASW. At 25 °C, the specific gravity and relative viscosity of ASW were 1.01 and 1.07, respectively; those of P-ASW were 1.04 and 1.14.

### *Recordings and analyses of gravity-dependent orientation*

Ni<sup>2+</sup>-immobilized *Paramecium* cells and KCN-immobilized sea urchin larvae were transferred, with experimental solutions to be tested, into a chamber made of a slide and coverslip and silicone rubber spacer (inner dimension 12 × 24 × 1 mm for *Paramecium* and 16 × 16 × 1 mm for sea urchin larvae) and kept air bubble-free without any particular sealant. The chamber was set on a horizontal microscope equipped with a rotating stage. After trapping immobilized specimens at the bottom or the top of the chamber (depending on the density of the medium), the chamber was rotated upside down, and the orientation motion during vertical movement due to gravity was recorded with a video camera (XC-77, Sony, Tokyo) and a videotape recorder. To avoid the hydrodynamic interactions between nearby moving objects, we chose organisms moving down (or up) far from neighbors (>1 mm, about 5 body lengths, apart). For

measuring the orientation angle, we selected recordings in which the orientation motion was observed in a single focal plane.

The orientation angle as a function of time ( $\theta$ ,  $t$ ) was measured directly on the video monitor. The rotational velocity as a function of orientation angle ( $d\theta/dt$ ,  $\theta$ ) was obtained as an average velocity ( $(\theta_{i+1} - \theta_i)/\Delta t$ ) at the angle of geometrical average ( $(\theta_i + \theta_{i+1})/2$ ) between every successive datum of inclination angle *versus* time.  $\beta$  in Equation 8 was obtained by nonlinear least-squares regression of the velocity data ( $d\theta/dt$ ,  $\theta$ ) to the equation

$$\frac{d\theta}{dt} = \beta \sin(\theta + \alpha), \quad (11)$$

where  $\alpha$  is a factor to adjust the angle between the morphologically defined fore-aft axis and the mechanically defined axis.

## Results

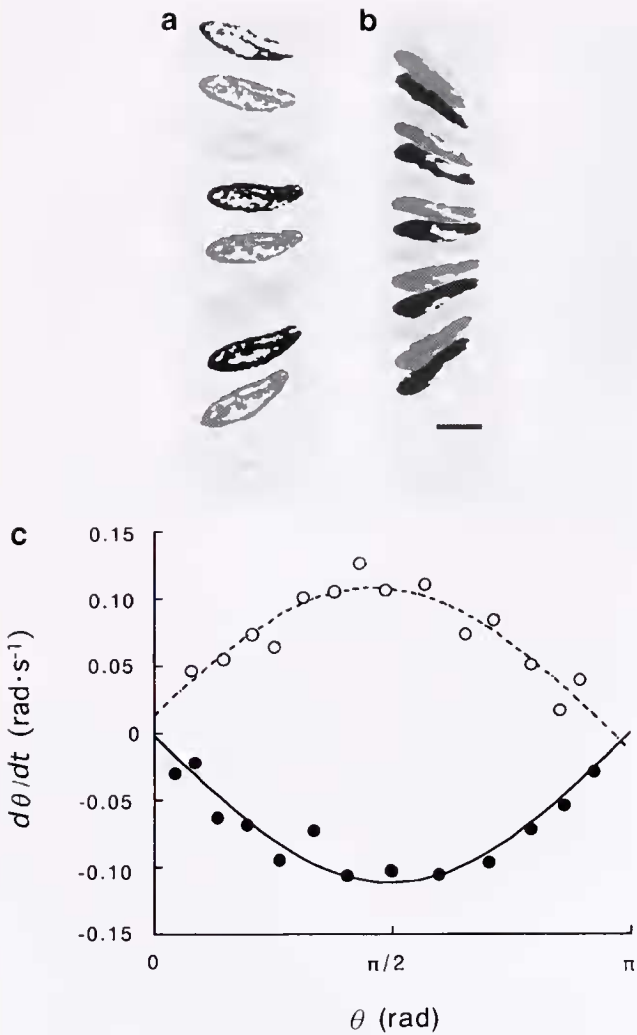
### *The drag-gravity model is the major mechanism of Paramecium*

When *Paramecium* was immobilized by Ni<sup>2+</sup>, it maintained an anterior-thinner cell shape. This shape was preserved in P-KCM as well as in KCM: cells showed no significant changes in axial length ( $162 \pm 17 \mu\text{m}$  [ $n = 30$ ] and  $163 \pm 16 \mu\text{m}$  [ $n = 21$ ],  $P = 0.64$ , for cells in KCM and P-KCM, respectively) or in maximum width ( $47.2 \pm 6.9 \mu\text{m}$  and  $46.5 \pm 4.7 \mu\text{m}$ ,  $P = 0.69$ ). Thus it is highly likely that rotational motion of the immobilized cell occurs with the same coefficient of resistance in both media.

Typical recordings of gravity-dependent orientation of immobilized paramecia in the hypo- and hyper-density media are shown in Figure 2a and b. In KCM ( $\rho < \rho_i$ ), paramecia oriented upwards during sinking due to gravity, whereas in P-KCM ( $\rho > \rho_i$ ) they oriented downwards during floating up. As shown in Figure 2c, plots of orientation rates ( $d\theta/dt$ ) against orientation angle ( $\theta$ ) fit well to the sinusoidal function of Equation 11. Values for  $\beta$  obtained by least-square regression were positive in the control hypo-density medium and negative in the hyper-density medium (Table 1). Negative values of  $\beta$  in the hyper-density medium indicate that the drag-gravity model is the major mechanism of mechanical gravitactic orientation in *Paramecium*.

### *Sea urchin larvae change the mechanical mechanism of gravitactic orientation during development*

When sea urchin larvae were treated with KCN, their cilia ceased beating and stood nearly perpendicular to the larval surface. The outer morphology of the larvae was observed to be well preserved in P-ASW as well as in ASW: for gastrulae, axial length was  $151 \pm 7.6 \mu\text{m}$  ( $n = 16$ ) and  $145 \pm 6.1 \mu\text{m}$  ( $n = 13$ ),  $P = 0.19$ , in ASW and P-ASW,



**Figure 2.** Typical examples of gravity-dependent orientation of  $\text{Ni}^{2+}$ -immobilized *Paramecium caudatum*. (a, b) Sequential images of gravity-dependent orientation of a cell in KCM (a) and of another in P-KCM (b), in which recorded images are superimposed at 1-s intervals and the time sequence of the motion is illustrated by cyclic change in tone (dark  $\rightarrow$  medium  $\rightarrow$  light). In each figure the anterior end of the cell is located to the right, and the gravity vector is towards the bottom of the figure. Scale bar, 0.1 mm. (c) Orientation rates ( $d\theta/dt$ ) as a function of the inclination angle ( $\theta$ ). Data from the cells shown in a (KCM) and b (P-KCM) are plotted with open and closed circles, respectively. Sinusoidal curves were obtained by the least-squares fitting to Equation 11.

respectively, and the maximum width was  $135 \pm 3.7 \mu\text{m}$  and  $132 \pm 5.7 \mu\text{m}$ ,  $P = 0.06$ ; for plutei, axial length was  $235 \pm 19 \mu\text{m}$  ( $n = 26$ ) and  $240 \pm 13 \mu\text{m}$  ( $n = 18$ ),  $P = 0.29$ , in ASW and P-ASW, respectively, and the maximum width was  $175 \pm 13 \mu\text{m}$  and  $175 \pm 12 \mu\text{m}$ ,  $P = 0.98$ . This may justify the common basis for drag coefficients in rotation in the different density media, as in *Paramecium*.

The gravity-dependent orientation of immobilized larvae is shown in Figure 3a to d, which demonstrates the clear difference between gastrula and pluteus. In ASW ( $\rho < \rho_l$ ), both gastrula and pluteus oriented upwards while sinking; in

hyper-density P-ASW, however, gastrula oriented downwards but pluteus upwards while floating up. As shown in Figure 3e and f, the orientation rate appears to be a sinusoidal function of the orientation angle; although data from larvae fitted less closely to Equation 11 than did those from *Paramecium*, this was probably due to the uncertainty in measuring the orientation angle of the larvae. We sometimes observed that larvae rotated slowly around the fore-aft axis during sedimentation. This slow axial rotation made it difficult to determine the fore-aft axis of the larvae.

As shown in Table 1, values of  $\beta$  obtained from gastrula larvae were positive in the control medium and negative in the hyper-density medium. Thus, in gastrulae as in *Paramecium*, the drag-gravity model is the major mechanism of passive gravitactic orientation. However, pluteus larvae have positive values of  $\beta$  both in the control and in the hyper-density medium (Table 1). The relatively weak dependency of  $\beta$  of plutei on the density of the external medium indicates that the gravity-buoyancy model is the major mechanism of passive gravitactic orientation in these larvae. These results indicate that sea urchin larvae change the mechanical mechanism of gravitactic orientation during development.

## Discussion

### Estimation of the contribution of the mechanical models in the gravitactic orientation

The Reynolds number of rotational motion ( $\text{Re}_r$ ) of the microorganisms is defined as

$$\text{Re}_r = \frac{l^2 \omega \rho}{\eta}, \quad (12)$$

where  $l$  is a characteristic body length and  $\omega$  is the angular velocity of rotation (Happel and Brenner, 1973). From the maximum velocity of rotation (*ca.*  $0.2 \text{ rad} \cdot \text{s}^{-1}$ , Table 1),  $\text{Re}_r$  of *Paramecium* or sea urchin larvae is calculated to be about  $2 \times 10^{-3}$ , which is sufficiently smaller than unity. This means that the linear assumption of Equation 7 (see the *Theory* section) is valid to formulate the rotational motion of these microorganisms.

The orientation torque generated as a result of the combination of the torque originating from different mechanical sources causes the passive orientation of the immobilized organisms. It is difficult to formulate the combination, because we know little about the density distribution within an organism and its geometrical asymmetry. The simplest assumption for the combination of the rotational torque is that  $G$ ,  $B$ , and  $H$  are located on the geometrical fore-aft axis of the organisms. This gives a sinusoidal function as a linear summation of the sinusoidal equations, each of which is deduced from the gravity-buoyancy and drag-gravity model, respectively. As a result, the orientation rate is given as

Table 1

Orientation rate ( $\beta$ ), in  $\text{rad} \cdot \text{s}^{-1}$ , measured in different density media

Organism	Normal medium			Percoll-containing medium		
	Mean $\pm$ SD	Range	<i>n</i>	Mean $\pm$ SD	Range	<i>n</i>
<i>Paramecium</i>	0.090 $\pm$ 0.033	0.043 – 0.183	23	-0.104 $\pm$ 0.058	-0.257 – -0.041	14
Sea urchin larvae						
Gastrula	0.140 $\pm$ 0.032	0.107 – 0.197	8	-0.120 $\pm$ 0.020	-0.150 – -0.090	7
Pluteus	0.157 $\pm$ 0.031	0.105 – 0.190	9	0.110 $\pm$ 0.013	0.097 – 0.137	7

$$\frac{d\theta}{dt} = (\beta_V + \beta_R) \sin \theta. \quad (13)$$

This simple linear assumption seems to be supported by the fact that  $\alpha$  in Equation 11 was calculated on average as nearly zero ( $0.00 \pm 0.26 \text{ rad}$  ( $n = 37$ ) for *Paramecium*,  $0.03 \pm 0.18$  ( $n = 15$ ) for gastrula, and  $0.06 \pm 0.21$  ( $n = 16$ ) for pluteus). Therefore, it is likely that the morphologically defined fore-aft axis almost coincides with the mechanically defined axis. According to the assumption above,  $\beta_S$  obtained in the different density media are given by

$$\beta_N = \frac{V\rho_i g L_G}{R\eta_N} + \frac{V(\rho_i - \rho_N) g L_H}{R\eta_N} \text{ and} \quad (14)$$

$$\beta_P = \frac{V\rho_i g L_G}{R\eta_P} + \frac{V(\rho_i - \rho_P) g L_H}{R\eta_P}, \quad (15)$$

where  $\beta_N$  is the maximum orientation velocity measured in the normal density ( $\rho_N$ ) medium (KCM or ASW) of the viscosity of  $\eta_N$ , and  $\beta_P$  is that measured in the hyper-density ( $\rho_P$ ) medium (P-KCM or P-ASW) of the viscosity of  $\eta_P$ . Equations 14 and 15 give  $L_H$ , the distance from B to H, as

$$L_H = \frac{\eta_N \beta_N - \eta_P \beta_P}{\rho_P - \rho_N} \cdot \frac{R}{Vg}, \quad (16)$$

and, thus,  $\beta_R$  and  $\beta_V$  are given by:

$$\beta_R = \frac{\rho_i - \rho_N}{\rho_P - \rho_N} \left( \beta_N - \frac{\eta_P}{\eta_N} \beta_P \right) \text{ and} \quad (17)$$

$$\beta_V = \beta_N - \beta_R. \quad (18)$$

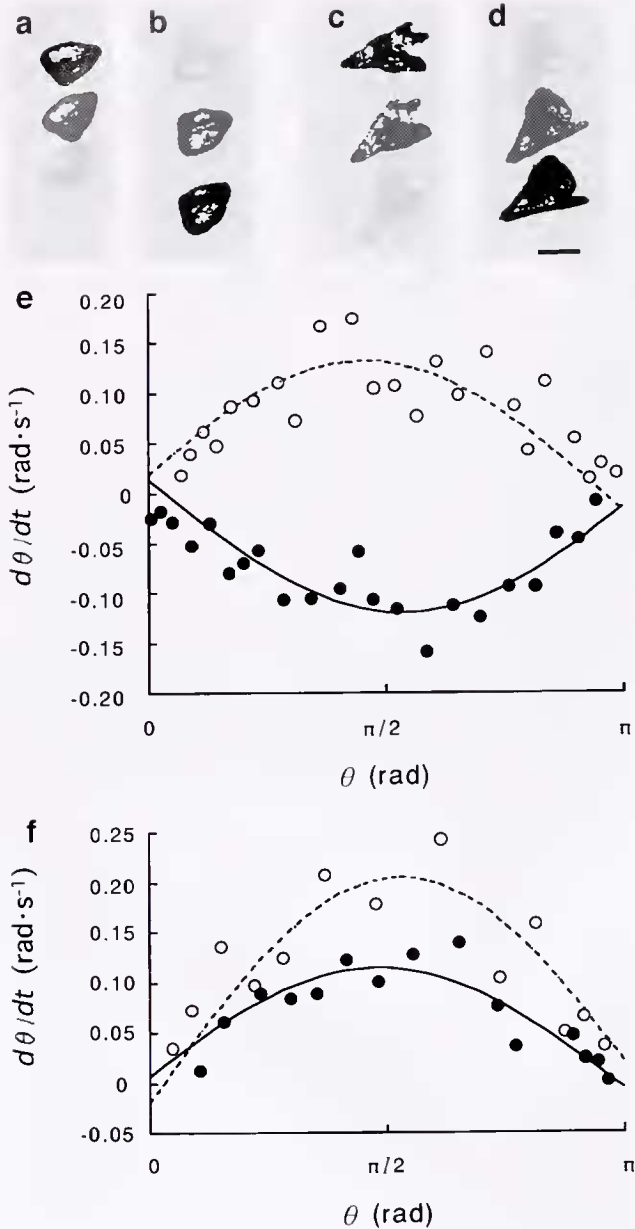
For *Paramecium*,  $\rho_N = 1.00$ ,  $\rho_P = 1.06$  and  $\rho_i = 1.03 \text{ g} \cdot \text{cm}^{-3}$  (Ooya *et al.*, 1992), and  $\eta_P/\eta_N = 1.53$ . For sea urchin larvae,  $\rho_N = 1.01$ ,  $\rho_P = 1.04$ , and  $\rho_i = 1.03$  and  $1.03 \text{ g} \cdot \text{cm}^{-3}$ , for gastrula and pluteus, respectively (values were obtained by sedimentation equilibrium experiments: data not shown), and  $\eta_P/\eta_N = 1.07$ . Using these values and  $\beta_N$  and  $\beta_P$  in Table 1, Equations 17 and 18 can be used to obtain values for the contribution of the two mechanisms to negative gravitaxis in normal-density medium. The upward orientation of *Paramecium* in KCM, corresponding to  $\beta_N = 0.09 \text{ rad} \cdot \text{s}^{-1}$ , is the result of an upward drag-gravity

component ( $\beta_R = 0.12 \text{ rad} \cdot \text{s}^{-1}$ ) combined with a smaller downward gravity-buoyancy component ( $\beta_V = -0.03 \text{ rad} \cdot \text{s}^{-1}$ ). The situation is similar for sea urchin gastrulae. The upward orientation with  $\beta_N = 0.14 \text{ rad} \cdot \text{s}^{-1}$  results from an upward drag-gravity component ( $\beta_R = 0.18 \text{ rad} \cdot \text{s}^{-1}$ ) combined with a small downward gravity-buoyancy component ( $\beta_V = -0.04 \text{ rad} \cdot \text{s}^{-1}$ ). However, the upward orientation of pluteus larvae with  $\beta_N = 0.16 \text{ rad} \cdot \text{s}^{-1}$  reflects a very different situation. The gravity-buoyancy component has reversed direction from downward to upward, and has increased to  $\beta_V = 0.13 \text{ rad} \cdot \text{s}^{-1}$ . The upward drag-gravity component has diminished greatly, to  $\beta_R = 0.03 \text{ rad} \cdot \text{s}^{-1}$ , so that it now makes only a small contribution to the upward orientation.

#### The mechanical property of *Paramecium*

There have been several investigations on the mechanical basis of the passive upward orientation of *Paramecium*. Most of them favored the gravity-buoyancy model as a major mechanism of gravitactic orientation. Fukui and Asai (1980) reported that Triton-treated immobilized cells oriented mostly upwards at the sedimentation equilibrium in sucrose density gradient. This upward orientation was evident in well-fed cells but not in starved cells. The upward-orienting posture was found under centrifugal forces in  $\text{Ni}^{2+}$ -immobilized cells in the isodensity medium (Taneda *et al.*, 1987) and also in the cells swimming at isopycnic level in the density gradient with Ficoll or Percoll (Kuroda and Kamiya, 1989). It was also reported that upward orientation was induced by centrifugal force effectively in the cells at the early culture phase but not in those at the late phase, which showed little or no gravitaxis. These results appear to conform with the conclusion that the upward orientation of *Paramecium* is strongly biased by the torque resulting from the higher density of the posterior part of the organism; the increased density is mainly due to the accumulation of food vacuoles (Fukui and Asai, 1985).

It should be noted, however, that the results of the sedimentation equilibrium experiments were ascribed only to the function of the gravity-buoyancy model and not to the contribution of the drag-gravity model, since  $F_H = 0$  with buoyancy artificially balanced with gravity. Furthermore, it



**Figure 3.** Typical examples of gravity-dependent orientation of KCN-immobilized sea urchin (*Hemicentrotus pulcherrimus*) larvae. (a–d) Sequential images of gravity-dependent orientation of the single different larvae at the gastrula (a and b) and the pluteus (c and d) stages. Movements of a larva in ASW (a and c) and of another in P-ASW (b and d) are shown at 3-s intervals in the same way as in Fig. 2a and b. In each figure the animal pole of the larva (leading end in forward swimming) is located to the right, and the gravity vector is towards the bottom of the figure. Scale bar, 0.1 mm (e, f) orientation rates ( $d\theta/dt$ ) as a function of the inclination angle ( $\theta$ ), measured from gastrula (e) and pluteus (f). In e, data from the gastrulae shown in a (ASW) and b (P-ASW) are plotted with open and closed circles, respectively. In f, data from the pluteus shown in c (ASW) and d (P-ASW) are plotted with open and closed circles, respectively. Sinusoidal curves were obtained by the least-squares fitting to Equation 11.

seems likely that the gravity-buoyancy component of the orientation torque might be enhanced in these experiments. Since the center of gravity would shift in relation to the

content and the distribution of organelles such as food vacuoles, it is probable that in the sedimentation equilibrium experiments, the intracellular distribution of the organelle was reorganized by gravity during long-lasting sedimentation of Triton-permeabilized cells through the sucrose density gradient (Fukui and Asai, 1980), or by a large centrifugal acceleration ( $100 \times g$ , Taneda *et al.*, 1987;  $300\text{--}400 \times g$ , Kuroda and Kamiya, 1989). This may result in accumulation of organelles in the rear part of the cell, and may cause upward orientation, even if the cells originally have a slightly top-heavy organelle distribution that gives a negative  $\beta_V$  as estimated above. These facts suggest that the results of previous experiments are still equivocal for the contribution of the drag-gravity model in the gravitactic orientation of *Paramecium*.

The evidence presented in the *Results*, on the contrary, indicate that the drag-gravity model makes a major contribution to generating a torque for the gravitactic orientation. Although the possibility of a minimal contribution cannot be ruled out, it is clear that the gravity-buoyancy model cannot solely explain the alteration of the sign of the rotational torque in the hyper-density medium. In addition, paramecia were observed in P-KCM to swim mostly downwards (data not shown). Swimming cells changed the net direction of their helical swimming trajectory gradually downwards and accumulated at the bottom of the chamber against the strong floating bias. Positive gravitaxis of *Paramecium* in the hyper-density medium can be explained by the drag-gravity model, not by the gravity-buoyancy model.

#### *Developmental changes in the mechanical property in sea urchin larvae*

In the present paper we demonstrated a change in the mechanical basis for gravitactic orientation during the development of sea urchin larvae: from the drag-gravity model in gastrulae to the gravity-buoyancy model in plutei. Gastrulae have a thicker posterior part, similar to that of *Paramecium*, which is required for the drag-gravity model to function. Plutei, on the other hand, have a thicker anterior part. Therefore they may orient the rear end upwards if the rotational torque is generated according to the drag-gravity model. This was not the case for plutei. Regardless of the remarkable fore-aft asymmetry in morphology, plutei obeyed the gravity-buoyancy model. Gravitactic orientation by different mechanisms was also revealed in the gravitactic swimming behavior of the larvae in P-ASW. In spite of the strong floating bias, gastrulae swam preferentially downwards (positive gravitaxis) and accumulated at the bottom of the chamber, whereas plutei swam upwards (negative gravitaxis) and accumulated at the top of the chamber (data not shown).

Mogami *et al.* (1988) found that sea urchin larvae change their gravitactic behavior during development. Larvae at the blastula stage to the early gastrula stage swim preferentially

upwards. This may be explained by a major upward drag-gravity component of orientation torque. The negative gravitactic behavior becomes less remarkable in prism larvae: they tend to swim in random directions independent of the gravity vector. This transient disappearance of gravitaxis may correspond to the alteration of the orientation mechanism revealed in the present paper. At the pluteus stage, larvae again show negative gravitaxis as they acquire the orientation mechanism with a major upward gravity-buoyancy component. A strong separation between the centers of gravity and buoyancy may develop in association with the growth of skeletal structures. Rudiments of spicules initiated in the early gastrula fully extend to give rise to the specific shape of the pluteus larva. The spicule is made of magnesian calcite with a density about three times higher than the average density (Okazaki and Inoué, 1976). As spicules grow, they may change the density distribution to shift the center of gravity toward the rear of the cell. If plutei hereafter maintained the rear-end-heavy mass distribution, they could maintain negative gravitactic behavior irrespective of pronounced morphological changes during the late larval stages.

Although the functional role of the drag-gravity model has been accepted in theory, it was not experimentally demonstrated in the orientation movement of organisms. In the present paper we present the first evidence that external geometry is actually important to the gravitactic behavior of aquatic microorganisms. The morphology-dependent interaction of the organisms with the external fluid seems to be more complicated than hypothesized in the *Theory* section of this paper. The slow axial rotation observed in sedimenting sea urchin larvae indicates a hydrodynamic coupling between translational and rotational motion (Happel and Brenner, 1973). Therefore, it is probable that the hydrodynamic coupling secondarily functions to drift the swimming direction upwards, as argued in previous researches (Winet and Jahn, 1974; Nowakowska and Grebecki, 1977).

In conclusion, the present study on the mechanical properties of gravitactic orientation in the gravity field demonstrates a relation between the morphology of microorganisms and their gravitactic behavior. This relationship might be instructive in researching cases of microbial gravitaxis whose mechanism is still disputed.

#### Acknowledgments

This study was carried out as a part of "Ground Research Announcement for Space Utilization" promoted by Japan Space Forum.

#### Literature Cited

- Bean, B.** 1984. Microbial geotaxis. Pp. 163–198 in *Membrane and Sensory Transduction*, G. Colombetti and F. Lenzi, eds. Plenum Press, New York.
- Chia, F.-S., J. Buckland-Nicks, and C. M. Young.** 1983. Locomotion of marine invertebrate larvae: a review. *Can. J. Zool.* **62**: 1205–1222.
- Degawa, M., Y. Mogami, and S. A. Baba.** 1986. Developmental changes in  $\text{Ca}^{2+}$  sensitivity of sea-urchin embryo cilia. *Comp. Biochem. Physiol.* **82A**: 83–90.
- Fenchel, T., and B. Finlay.** 1984. Geotaxis in the ciliated protozoan *Loxodes*. *J. Exp. Biol.* **110**: 17–33.
- Fenchel, T., and B. Finlay.** 1986. The structure and function of Muller vesicles in loxodid ciliates. *J. Protozool.* **33**: 68–76.
- Fukui, K., and H. Asai.** 1980. The most probable mechanism of the negative geotaxis of *Paramecium caudatum*. *Proc. Jpn. Acad.* **56(B)**: 172–177.
- Fukui, K., and H. Asai.** 1985. Negative geotactic behavior of *Paramecium caudatum* is completely described by the mechanism of buoyancy-oriented upward swimming. *Biophys. J.* **47**: 479–482.
- Happel, J., and H. Brenner.** 1973. *Low Reynolds Number Hydrodynamics*. Noordhoff International Publishing, Leyden.
- Kuroda, K., and N. Kamiya.** 1989. Propulsive force of *Paramecium* as revealed by the video centrifuge microscope. *Exp. Cell Res.* **184**: 268–272.
- Machemer, H., and R. Bräucker.** 1992. Gravitoreception and gravireponses in ciliates. *Acta Protozool.* **31**: 185–214.
- Machemer, H., S. Machemer-Röhnisch, R. Bräucker, and K. Takahashi.** 1991. Gravitaxis in *Paramecium*: theory and isolation of a physiological response to the natural gravity vector. *J. Comp. Physiol. A* **168**: 1–12.
- Machemer-Röhnisch, S., U. Nagel, and H. Machemer.** 1999. A gravity-induced regulation of swimming speed in *Euglena gracilis*. *J. Comp. Physiol.* **185**: 517–522.
- Mogami, Y., C. Oohayashi, T. Yamaguchi, Y. Ogiso, and S. A. Baba.** 1988. Negative geotaxis in sea urchin larvae: a possible role of mechanoreception in the late stages of development. *J. Exp. Biol.* **137**: 141–156.
- Nowakowska, G., and A. Grebecki.** 1977. On the mechanism of orientation of *Paramecium caudatum* in the gravity field. II. Contributions to a hydrodynamic model of geotaxis. *Acta Protozool.* **16**: 359–370.
- Okazaki, K., and S. Inoué.** 1976. Crystal property of the larval sea urchin spicule. *Dev. Growth Differ.* **18**: 413–434.
- Ooya, M., Y. Mogami, A. Izumi-Kurotani, and S. A. Baba.** 1992. Gravity-induced changes in propulsion of *Paramecium caudatum*: a possible role of gravireception in protozoan behaviour. *J. Exp. Biol.* **163**: 153–167.
- Roberts, A. M.** 1970. Geotaxis in motile micro-organisms. *J. Exp. Biol.* **53**: 687–699.
- Taneda, K., S. Miyata, and A. Shiota.** 1987. Geotactic behavior in *Paramecium caudatum* II. Geotaxis assay in a population of the specimens. *Zool. Sci.* **4**: 789–795.
- Verworn, M.** 1889. *Psychophysiologische Protistenstudien*. Gustav Fischer, Jena. (Cited by Machemer and Bräucker, 1992.)
- Vogel, S.** 1994. *Life in Moving Fluids: The Physical Biology of Flow*, 2nd ed. Princeton University Press, Princeton, NJ.
- Winet, H., and T. L. Jahn.** 1974. Geotaxis in protozoa. I. A propulsion gravity model for *Tetrahymena* (Ciliata). *J. Theor. Biol.* **46**: 449–465.